

Claims

1. Method for increasing the production of cysteine, glutathione and methionine, and of sulphur derivatives thereof, by plant cells and plants, the
5 said method consisting in overexpressing an SAT in plant cells and plants containing the said plant cells.
2. Method according to claim 1, characterized in that the SAT which is overexpressed in plant cells is a cysteine-sensitive SAT.
- 10 3. Method according to claim 2, characterized in that the SAT is a plant SAT or a native SAT of bacterial origin.
4. Method according to claim 1, characterized in that the SAT which is overexpressed in
15 plant cells is a cysteine-insensitive SAT.
5. Method according to claim 4, characterized in that the SAT is a plant SAT or an SAT of bacterial origin, or a mutated plant SAT, rendered cysteine-insensitive by mutagenesis.
- 20 6. Method according to one of claims 1 to 5, characterized in that the SAT is overexpressed in the cytoplasm of plant cells.
7. Method according to claim 6, characterized in that the SAT is an SAT of bacterial
25 origin.
8. Method according to claim 6, characterized in that the SAT is a plant cytoplasmic SAT, in particular from *Arabidopsis thaliana*.

9. Method according to claim 8,
characterized in that the SAT is SAT3 which is
represented by SEQ ID NO 1.

10. Method according to claim 6,
5 characterized in that the SAT is a non-cytoplasmic
plant SAT from which has been removed its signal(s) for
addressing to cellular compartments other than the
cytoplasm.

11. Method according to claim 10,
10 characterized in that the SAT is SAT1' which is
represented by SEQ ID NO 2.

12. Method according to one of claims 1 to
5, characterized in that the SAT is overexpressed in
mitochondria.

13. Method according to claim 12,
15 characterized in that the SAT is overexpressed in the
cytoplasm in the form of a signal peptide/SAT fusion
protein, the mature functional SAT being released
inside mitochondria.

14. Method according to claim 13,
20 characterized in that the mitochondrial addressing
signal peptide consists of at least one signal peptide
from a natural plant protein which is located in
mitochondria, such as for example, the SAT1 signal
25 peptide which is represented by amino acids 1 to 63 in
SEQ ID NO 3.

15. Method according to claim 13,
characterized in that the SAT is a mitochondrial SAT of
plant origin, in particular from *Arabidopsis thaliana*.

16. Method according to claim 15,
5 characterized in that the SAT is SAT1 which is
represented by SEQ ID NO 3.

17. Method according to claim 6,
characterized in that the SAT is overexpressed in
chloroplasts of plant cells.

10 18. Method according to claim 17,
characterized in that the SAT is overexpressed in
chloroplasts by integration, into chloroplast DNA of
plant cells, of a chimeric gene comprising a DNA
sequence encoding the said SAT, under the control of 5'
15 and of 3' regulatory elements which are functional in
chloroplasts.

19. Method according to claim 17,
characterized in that the SAT is overexpressed in the
cytoplasm in the form of a transit peptide/SAT fusion
20 protein, the mature functional SAT being released
inside chloroplasts.

20. Method according to claim 19,
characterized in that the SAT is homologous with the
transit peptide.

25 21. Method according to claim 20,
characterized in that the SAT is a chloroplast SAT of
plant origin, in particular from *Arabidopsis thaliana*.

22. Method according to claim 21,
characterized in that the SAT is SAT2 or SAT4 which are
represented by SEQ ID NO 5 or NO 6, respectively.

23. Method according to claim 19,
5 characterized in that the SAT is heterologous with the
transit peptide.

24. Method according to claim 13,
characterized in that the SAT is a cytoplasmic SAT of
plant origin or an SAT of bacterial origin, as defined
10 in one of claims 3 to 5 or 9 to 11.

25. Method according to either of claims 23
and 24, characterized in that the transit peptide is a
transit peptide from another protein which is located
in plastids.

15 26. Method according to claim 25,
characterized in that the transit peptide consists of a
plant EPSPS transit peptide or a plant RuBisCO ssu
transit peptide.

27. Method according to either of claims 25
20 and 26, characterized in that the transit peptide
comprises a transit peptide from a plant protein which
is located in plastids, and, between the C-terminal
portion of the transit peptide and the N-terminal
portion of the SAT, a portion of sequence from the
25 mature N-terminal region of a protein which is located
in plastids.

28. Method according to claim 27,
characterized in that the portion of sequence comprises

generally less than 40 amino acids from the N-terminal portion of the mature protein, preferably less than 30 amino acids, more preferably between 15 and 25 amino acids.

5 29. Method according to either of claims 27 and 28, characterized in that the transit peptide comprises, between the C-terminal portion of the N-terminal portion of the mature protein and the N-terminal portion of the SAT, a second transit peptide
10 from a plant protein which is located in plastids.

 30. Method according to claim 29, characterized in that the transit peptide is an optimized transit peptide (OTP) made by fusing a first transit peptide with a portion of sequence from the
15 mature N-terminal region of a protein located in plastids, which is fused with a second transit peptide.

 31. Transit peptide/SAT fusion protein, characterized in that the SAT is heterologous with the transit peptide.

20 32. Fusion protein according to claim 31, as defined in claims 24 to 30.

 33. Nucleic acid sequence encoding a transit peptide/SAT fusion protein according to either of claims 31 and 32.

25 34. Chimeric gene comprising a coding sequence as well as heterologous 5' and 3' regulatory sequences, which are able to function in a host organism, characterized in that the coding sequence

comprises at least one nucleic acid sequence which encodes an SAT.

35. Chimeric gene according to claim 34, characterized in that the host organism is chosen from
5 bacteria, for example *E. coli*, yeasts, in particular of the genera *Saccharomyces*, *Kluyveromyces* or *Pichia*, fungi, in particular *Aspergillus*, baculoviruses, or plant cells and plants.

36. Chimeric gene according to claim 35,
10 characterized in that the host organism is a plant cell or a plant which contains it .

37. Chimeric gene according to claim 36, characterized in that the 5' regulatory element comprises regulatory sequences which are promoters in
15 plant cells and plants, and are chosen from promoters which are expressed in plant leaves, constitutive promoters, or light-dependent promoters of bacterial, viral or plant origin.

38. Chimeric gene according to claim 36,
20 characterized in that the 5' regulatory element comprises regulatory sequences which are promoters in plant cells and plants, and are chosen from seed-specific promoters.

39. Chimeric gene according to claim 38,
25 characterized in that the promoter is chosen from the promoters for napin, phaseolin, glutenin, zein, helianthinin, albumin and oleosin.

40. Chimeric gene according to one of claims 34 to 39, characterized in that the nucleic acid sequence which encodes an SAT encodes an SAT as defined in claims 2 to 30.

5 41. Chimeric gene according to one of claims 34 to 39, characterized in that the nucleic acid sequence which encodes an SAT is the nucleic acid sequence according to claim 33.

42. Cloning and/or expression vector for
10 transforming a host organism, characterized in that it contains at least one chimeric gene as defined according to one of claims 34 to 41.

43. Method of transforming host organisms, characterized in that at least one nucleic acid
15 sequence according to claim 33, or a chimeric gene according to one of claims 34 to 41, is integrated into the genome of the said host organism.

44. Method according to claim 43, by means of the vector according to claim 42.

20 45. Method according to either of claims 43 and 44, characterized in that the host organism is chosen from bacteria, for example *E. coli*, yeasts, in particular of the genera *Saccharomyces*, *Kluyveromyces* or *Pichia*, fungi, in particular *Aspergillus*,
25 baculoviruses, or plant cells and plants.

46. Method according to claim 45, characterized in that the host organism is a plant cell or a plant which contains it.

47. Method according to claim 46,
characterized in that the plant is regenerated from a
transformed plant cell.

48. Method according to claim 47,
5 characterized in that the host organism is a
monocotyledonous plant, in particular chosen from
cereals, sugar cane, rice and maize, or a
dicotyledonous plant, in particular chosen from
tobacco, soybean, rape, cotton, beet and clover.

10 49. Transformed host organism, characterized
in that it comprises at least one nucleic acid sequence
according to claim 33, or a chimeric gene according to
one of claims 34 to 41.

50. Host organism according to claim 49,
15 characterized in that it is obtained by the method
according to one of claims 43 to 48.

51. Plant cell, characterized in that it
comprises at least one nucleic acid sequence according
to claim 33, or a chimeric gene according to one of
20 claims 34 to 41.

52. Genetically modified plant,
characterized in that it comprises at least one plant
cell according to claim 51.

53. Plant according to claim 52,
25 characterized in that the plant is regenerated from a
plant cell according to claim 51.

54. Genetically modified plant,
characterized in that it is derived from the culture

and/or crossing of regenerated plants, according to claim 53.

55. Genetically modified plant according to one of claims 52 to 54, characterized in that it is a
5 monocotyledonous plant, in particular chosen from cereals, sugar cane, rice and maize, or a dicotyledonous plant, in particular chosen from tobacco, soybean, rape, cotton, beet and clover.

56. Genetically modified plant according to
10 one of claims 52 to 55, characterized in that it comprises other genes of interest.

57. Genetically modified plant according to claim 56, characterized in that it comprises at least one other gene which modifies the content and quality
15 of the proteins of the said plant, in particular in the leaves and/or seeds.

58. Genetically modified plant according to either of claims 56 and 57, characterized in that the gene encodes a protein enriched in sulphur-containing
20 amino acids.

59. Seeds of genetically modified plants according to one of claims 52 to 58.